Liquid chromatography-high-resolution mass spectrometry analysis of erectile dysfunction drugs and their analogues in food products

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Abstract

The presence of erectile dysfunction (ED) drugs in adulterated dietary supplements, mainly in pharmaceutical dosage forms, is frequently addressed in the literature. Little attention is given to food products despite their increasing adulteration trend. To address this knowledge gap targeted, suspected-target, and non-targeted strategies were utilised to analyse ED drugs and their analogues in powdered drink mix (PDM), honey, jelly, hard candy, and sugar-coated chewing gum using liquid chromatography-high-resolution mass spectrometry (LC-HRMS). The method was optimised and validated using 23 target analytes, representing different ED drugs with structural similarities. The modified quick, easy, cheap, effective, rugged, and safe (QuEChERS) extraction exhibited insignificant matrix effect (ME) within -9.2%–8.8% and provided complete coverage of target analytes with acceptable extraction recovery (RE) within 75.5%–123.9%, except for carbodenafil in the PDM matrix. Based on the ME and RE performance, the analytical method was validated to analyse 25 food samples that claimed to enhance male sexual performance. The method exhibited good specificity and linearity with a limit of detection within 10–70 ng/mL and limit of quantification of 80 ng/mL. Similarly, the accuracy and precision were satisfactory within 77.4%–122.0% and <16.7%RSD, respectively. The LC-HRMS targeted analysis, together with suspected-target and non-targeted screenings, identified and detected ten ED drugs from 24 food samples. The modified QuEChERS extraction with LC-HRMS-based method was demonstrated to be universally applicable to various food products, covering an extensive range of known and potentially novel ED drugs, which is valuable for routine casework.

Keywords: Adulterated food products, Erectile dysfunction drugs, LC-HRMS, Non-targeted screening, Suspected-target screening

1. Introduction

Erectile dysfunction (ED) drugs are currently in high demand due to the immense success of sildenafil, vardenafil, and tadalafil [1]. Unfortunately, these phosphodiesterase 5 inhibitors are often deliberately added into various dietary supplements to deliver the desired efficacy, despite their health risks to consumers [2]. Worse, they usually contain analogues of the approved drugs [3, 4], presenting significant health and life-threatening risks, attributed by their unknown safety and toxicological profiles [5, 6]. Lately, these unscrupulous manufacturers have been finding ways to conceal the adulterants within complex matrices such as food products, which may hinder detection and circumvent the law.

Liquid chromatography (LC) coupled to mass spectrometry (MS), particularly in tandem mode is commonly used to determine ED drugs in adulterated products due to its superior specificity, sensitivity, and the ability to separate multiple analytes from complex matrices [7]. The electrospray ionisation (ESI) technique in positive mode indubitably identified the ED drugs due to their physical and chemical properties [8]. However, this technique has many drawbacks, mostly caused by matrix effect (ME), leading to various errors, especially in the quantification process [9]. Several strategies have been proposed to mitigate the ME, covering three broad categories of (1) sample extraction, (2) ionisation technique, and (3) chromatographic separation [10].

The determination of ED drugs as adulterants in dietary supplements, particularly those in pharmaceutical dosage forms, is frequently discussed in the literature. Most of these studies used dilute and shoot (D&S) procedure with either acetonitrile [11] or methanol [12], including combining either solvent with ultrapure water [13]. The D&S is undoubtedly straightforward, simple, and quick; where no sample clean-up is involved [7]. Unfortunately, it is prone to matrix interference that may affect ED drugs analysis. Only a small number of studies employed extraction techniques such as liquid-liquid extraction (LLE) [14-16] and solid-phase extraction (SPE) [17, 18].

An up-to-date search found only a few studies focused exclusively on ED drugs determination in food products, for instance, Chinese tonic liquor [19] and instant coffee premix [20-22]. Other studies [23-25] included them as supplementary samples, with little to no information on matrix-specific validation, particularly on ionisation suppression or enhancement. This study's primary objective is to analyse ED drugs and their analogues in selected food products through targeted, suspected-target, and non-targeted strategies using liquid chromatography-high-resolution mass spectrometry (LC-HRMS). The ME and extraction recovery (RE) of 23 targeted ED drugs were evaluated in positive ESI mode and then validated for each food matrix. Subsequently, the method was applied to analyse 25 food samples that claimed to enhance male sexual performance.

2. Materials and methods

2.1. Chemicals and reagents

A total of 23 reference standards of ED drugs (purity ≥ 98%) were purchased from TLC Pharmaceutical Standards Ltd. (Aurora, Ontario, Canada). They were as follows: (1) desmethylcarbodenafil (99.7%), (2) carbodenafil (99.2%), (3) N-desethylacetildenafil (99.8%), (4) acetildenafil (99.2%), (5) hydroxyvardenafil (99.8%), (6) dimethylacetildenafil (99.8%), (7) vardenafil (99.3%), (8) sildenafil (99.3%), (9) homosildenafil (99.3%), (10) dimethylsildenafil (99.7%), (11) propoxyphenyl-hydroxyhomosildenafil (99.1%), (12) udenafil (99.1%), (13) propoxyphenyl-sildenafil (99.4%), (14) hydroxythiovardenafil (98.8%), (15) tadalafil (99.9%), (16) mirodenafil (99.9%), (17) mutaprodenafil (99.0%), (18) thiosildenafil (99.8%), (19) thiohomosildenafil (99.3%), (20) dithiodesmethylcarbodenafil (99.3%), (21) thiodimethylsildenafil (99.9%), (22) propoxyphenyl-thiohydroxyhomosildenafil (99.9%), and (23) propoxyphenyl-thiodimethylsildenafil (98.8%).

Chem-Supply Pty Ltd. (Gillman, SA, Australia) supplied the methanol and acetonitrile of LC-MS grade; while the vendor for formic acid of LC-MS grade and ammonium formate of analytical grade was Sigma Aldrich Pty Ltd. (Castle Hill, NSW, Australia). A Sartorius arium® pro ultrapure water system (Goettingen, Germany) dispensed the ultrapure water (18.2 MΩ-cm). LECO Australia Pty Ltd. (Castle Hill, NSW, Australia) provided the EN 15662 quick, easy, cheap, effective, rugged, and safe (QuEChERS) extraction salt.

2.2. Standard solution preparation

The stock solution of each reference standard was prepared in methanol at 1 mg/mL and stored in the dark at 4°C. A mixture of all 23 reference standards was freshly prepared for each analysis from the stock solutions by further dilution in methanol to produce 25 µg/mL of working solution.

2.3. Sample collection and storage

A total of 25 distinct food samples were obtained from Malaysia (23 samples) and Australia (two samples); in the form of powdered drink mix (PDM, 16 samples), honey (HNY, four samples), jelly (JLY, two samples), hard candy (HCD, two samples), and sugar-coated chewing gum (CWG, one sample). These products were selected based on their brand names, label claims, images, herbal ingredients, or advertising materials with connotations to male sexual performance. The Pharmacy Enforcement Division, Ministry of Health Malaysia, kindly donated most of these samples, which were confiscated by the pharmacy enforcement officers at the international airport (three samples) and international seaport (six samples), as well as from routine market surveillance activities (12 samples). The remaining samples were purchased from various online retail stores based in Malaysia and Australia (two samples each).

The samples were kept in separate plastic zip-lock bags and stored in an airtight container in the dark. Blank matrices of each food product, analysed to be free from any analyte of interests, were sourced from a local supermarket in Australia and used for method optimisation and validation. Table 1 outlines each food product's compositions used as the blank matrix based on the products' label.

2.4. Sample extraction procedures

The initial weight of each sample was recorded based on the recommended intake specified on its label. PDM and HNY were mixed by shaking and pressing the package content, respectively, and taken directly from their sachets. JLY was homogenised, while HCD and CWG were ground into fine granules using mortar and pestle before the sample extraction procedures. Initially, 100 mg of the mixed, homogenised, or finely granulated sample was weighed in a polypropylene tube and then added with 2.5 mL of acetonitrile and 2.5 mL of methanol; with subsequent vortex mixing for 1 min, sonication for 20 min, and centrifugation for 5 min at 2500 × *g*. The resulting solution was then transferred into another polypropylene tube prefilled with half a sachet of the QuEChERS extraction salt (2 g magnesium sulphate, 0.5 g sodium chloride, 0.5 g trisodium citrate dihydrate, and 0.25 g disodium hydrogen citrate sesquihydrate) for the extraction procedure with 1-min vortex mixing and 5-min centrifugation at 2500 × *g*, successively. The upper layer was then filtered and diluted at 1:10 dilution with methanol for LC-HRMS analysis. The blank food matrices were treated in the same manner as the steps described for the sample analysis.

2.5. LC-HRMS conditions and data analysis

An Agilent Technologies (Santa Clara, CA, USA) 1290 Infinity II LC system coupled to an Agilent Technologies 6510 quadrupole time-of-flight MS (QTOF-MS) was used in this study. In brief, the chromatographic separation was carried out using a reverse-phase high-performance LC column from Merck KGaA (Darmstadt, Germany) Chromolith® High-Resolution RP-18 end-capped (100 × 4.6 mm, 2.0 µm). The injection volume was set at 5 µL with the column and

autosampler compartment temperatures maintained at 20°C and 10°C, respectively. The mobile phases consisted of 10 mM ammonium formate in ultrapure water (solvent A) and acetonitrile (solvent B), acidified with 0.1% of formic acid. The gradient elution was set as follows: 5% B for 0–1 min; 5%–25% B for 1–2 min; 25%–50% B for 2–32 min; 50%–95% B for 32–33 min; and 95% B for 33–34 min at 0.4 mL/min. The elution was immediately returned to the initial gradient at 34.01 min for 6 min at 1 mL/min with post-run equilibration kept at 0.4 mL/min, 5 min before the next injection.

The QTOF-MS was operated in positive ESI mode with the following experimental parameters: 300°C for gas temperature, 12 L/min for drying gas flow, 32 psig for nebuliser pressure, 3500 V for capillary voltage, 175 V for fragmentor voltage, 65 V for skimmer voltage, and 750 V for octopole 1 radio frequency peak-to-peak voltage (OCT 1 RF Vpp). Simultaneous MS and tandem MS experiments within a mass-to-charge range of *m/z* 100–1100 were performed using auto MS/MS mode. The collision-induced dissociation experiments were conducted at fixed collision energies (CEs) of 10, 20, and 40 eV in a separate scan using nitrogen as the collision gas. The reference mass solution, containing purine (*m/z* 121.050873) and hexakis (1H, 1H, 3H-tetrafluoropropoxy) phosphazine (*m/z* 922.009798), was continually infused at a steady pressure of 5 psig throughout the chromatographic run.

All qualitative and quantitative data were processed with targeted analysis, together with suspected-target and non-targeted screenings, developed previously [20, 26] using Agilent Technologies Mass Hunter workstation software

version B.07.00, Mass Hunter qualitative analysis software version B.07.00, with Personal Compound Database and Library (PCDL) manager software version B.04.00. The suspected-target screening employed a PCDL library of 95 ED drugs and their analogues, including the 23 target analytes. The non-targeted screening through top-down and bottom-up approaches was utilised to flag novel analogues of ED drugs based on target analytes' common fragmentation patterns.

2.6. Sample extraction optimisation

The sample extraction was evaluated based on the ME and RE of each target analyte at low (0.1 µg/mL); medium (0.4 µg/mL); and high (1 µg/mL) quality control (QC) levels, each analysed in triplicate, following the recommended procedures [9, 27]. Each blank food matrix was assessed by preparing three sets of standards as follows: (1) standards in neat solution; (2) post-extraction spiked matrix (matrix-matched standards); and (3) pre-extraction spiked matrix.

The ME was evaluated based on the post-extraction addition method, by comparing the slopes of the post-extraction spiked matrix versus those of the standards in neat solution, expressed in Eq. 1. The calibration curves were constructed using the QC analytes' concentrations at three levels of matrix dilutions of 1:2, 1:10, and 1:100. The percentage of ME was then categorised for each target analyte following the set criteria of insignificant (0% to ±10%), acceptable (±10% to ±20%), moderate (±20% to ±50%), and severe (< -50% or > +50%), where a positive value indicates ionisation enhancement. In contrast, a negative value indicates ionisation suppression.

$$ME\ (\%) = \left[\frac{Slope_{post\text{-}extraction\ spiked\ matrix}}{Slope_{standards\ in\ neat\ solution}} - 1\right] \times 100 \qquad \text{(Eq. 1)}$$

The RE was determined by comparing the peak areas of the protonated molecule ($[M+H]^+$) precursor ion from the pre-extraction spiked matrix versus those of the post-extraction spiked matrix at the same QC level, expressed in Eq. 2. The mean RE was expressed in percentage at low, medium, and high QC levels with an acceptable value of ±25%.

$$RE\ (\%) = \frac{Peak\ area_{pre\text{-}extraction\ spiked\ matrix}}{Peak\ area_{post\text{-}extraction\ spiked\ matrix}} \times 100 \qquad \text{(Eq. 2)}$$

2.7. Analytical method validation

The analytical method was validated for specificity, linearity, limit of detection (LOD), and limit of quantification (LOQ), according to the established guideline [28]. The accuracy and precision were evaluated for each target analyte, in each of the blank food matrices, at low, medium, and high QC levels, following the recommended procedures [27]. All validation parameters were analysed in triplicate.

Each target analyte's specificity was determined based on the chromatographic separation, the high-resolution mass of the ($[M+H]^+$), and the extent of matrix interferences. The presence of two product ions, corresponding to each of the target analytes, was then established from the tandem MS experiment. Furthermore, the average intensity ratio between the first and the second product

ion at averaged CEs was compared to those obtained from the matrix-matched standards within ±30%, hence confirming the target analytes' identities.

The linearity was determined based on the coefficient of determination (r^2) of a six-point external calibration curve in neat solutions, constructed using the peak areas of the $[M+H]^+$ precursor ion, versus their concentrations within the expected range of target analytes in adulterated food products. The regression equation was then used to calculate the QC analytes and samples concentrations.

The LOD was determined experimentally by gradually reducing the concentration of target analytes by 10 ng/mL, starting from 100 ng/mL and down to 10 ng/mL. The LOD was then established at the lowest concentration of target analyte that can be reliably identified as defined in the specificity assessment, while the LOQ was set at the lowest concentration of the external calibration curve with acceptable accuracy and precision.

The accuracy was established at three QC levels where all target analytes were spiked into each of the extracted blank food matrices. Peak areas of the $[M+H]^+$ precursor ion were fitted to the regression equation of the external calibration curve to determine each target analyte's concentration. The observed concentration of target analyte versus the expected concentration at the same QC level was expressed as a percentage of accuracy with an acceptable value of ±25%. The precision was established using the same QC analytes at intra-day for repeatability and inter-day for intermediate precision. The peak areas of the

$[M+H]^+$ precursor ion were then expressed as a percentage of relative standard deviation (%RSD) with an acceptable value of <20%RSD.

3. Results and discussion

3.1. Method development and optimisation

The simultaneous analysis of ED drugs with diverse chemical structures from complex matrices, such as food products, can be challenging for forensic drug testing laboratories. The distinct physical and chemical properties of each ED drug may additionally hinder the extraction efficiency. In this study, 23 target analytes were selected to represent different ED drugs based on their structural similarities. The ME and RE of these analytes in five blank food matrices, i.e. PDM, HNY, JLY, HCD, and CWG, were initially evaluated using different extraction procedures. The relationship between matrix dilution and ionisation suppression or enhancement was also investigated at 1:2, 1:10, and 1:100 while maintaining the target analytes concentration at three QC levels.

During the early stage of method development, D&S, LLE, and SPE procedures were compared to assess their performance for the selected food matrices. However, they often led to non-detection, also moderate to severe ME of several target analytes. Specific ED drugs, particularly those with sulphur-containing pyrazolopyrimidine-7-thione and imidazotriazine-4-thione, as well as acetyl-bonded pyrazolopyrimidine-7-one, often resulted in poor RE. As these extraction procedures were previously applied for adulterated products in pharmaceutical dosage forms, they may not be compatible with complex matrices such as food products.

The modified QuEChERS extraction, on the contrary, resulted in acceptable RE for all target analytes in each of the food matrices within 75.5%–123.9%, except for carbodenafil in the PDM matrix at low (43.5%), medium (54.3%), and high (49.7%) QC levels (supplementary Table S1). Besides exhibiting acceptable RE for almost all target analytes in the selected food matrices, the modified QuEChERS extraction is superior, substantiated by acceptable and insignificant MEs even at the lowest level of 1:2 matrix dilution. The ME was minimised to insignificant percentages with increasing matrix dilutions from 1:2 to 1:100. These results demonstrated that matrix dilution is an effective strategy to mitigate the ME for each food matrix. However, higher levels of matrix dilutions may reduce the overall analytical method sensitivity, and therefore, should be carefully evaluated and selected to achieve accurate and precise quantification. Therefore, the method was validated at 1:10 matrix dilution owing to the insignificant ME within -9.2%–8.8%, covering each target analyte in all food matrices (supplementary Table S2).

3.2. Analytical method validation

The specificity (chemical formula, retention time, the accurate mass of $[M+H]^+$ precursor ion and product ions), linearity (r^2), and sensitivity results (LOD) are shown in Table 2. The optimised chromatographic separation and the HRMS data of the $[M+H]^+$ precursor ion established each target analyte specificity, while their identities were confirmed by the presence of two product ions from the tandem MS experiments. Interferences and carry-over effects, specifically from the extracted blank food matrices, were not observed at the retention time of target analytes and in subsequent analysis, respectively. The linearity of each target

analyte was verified by r^2 of >0.9960 within the selected range of 0.08–1.2 µg/mL. The LOD was established experimentally between 10 and 70 ng/mL, while the LOQ was set at 80 ng/mL for all target analytes.

The accuracy, repeatability, and intermediate precision results are presented in Tables S3–S5 (supplementary data), respectively. The accuracy was satisfactory within 77.4%–122.0% for all target analytes at low, medium, and high QC levels. The results show that the linear relationship between target analytes' signals and their concentrations in matrix-matched standards correlates well with that between target analytes' signals and their concentrations in neat solution. Based on these findings, it is possible to quantify all target analytes using the external calibration curve for all the food matrices. Similarly, the precision was acceptable with the %RSD of <16.7%. The repeatability and intermediate precision for all blank food matrices were calculated within 0.1%–9.5% and 0.1%–16.7% of RSD, respectively, at all QC levels.

3.3. Analysis of ED drugs in food samples

Table 3 compiles the analysis results of the 25 food samples. The suspected-target screening matched 24 samples with ten ED drugs from the PCDL library. The tandem MS and retention time matching through the targeted analysis subsequently confirmed the identity of eight target analytes from 23 samples. The remaining two suspected analytes, i.e. propoxyphenyl-dimethylsildenafil and nortadalafil, were detected from samples HNY004 and HCD002, respectively. Sample HNY004 also comprised other target analytes such as propoxyphenyl-thiodimethylsildenafil, thiodimethylsildenafil, and dimethylsildenafil. The

interrogations of MS and tandem MS spectra using top-down and bottom-up approaches of the non-targeted screening returned insignificant signals, indicating the absence of potentially novel analogues of ED drugs from all 25 samples, thus, confirming PDM009 as the only non-adulterated sample.

The suspected-target screening can be depicted using sample HCD002, displayed in Fig. 1. Analysis of the total ion chromatogram with the "Find Compounds by Formula (FBF)" technique, using the PCDL library, returned one peak which matched to nortadafil at *m/z* of 376.1288, with a mass error of -1.1 ppm (Fig. 1A). The observed mass, isotopic abundance distribution, and isotopic spacing from the full MS scan (Fig. 1B) were also comparable to those theoretically predicted from the chemical formula of nortadalafil ($C_{21}H_{17}N_3O_4$). Nortadalafil is an analogue of an approved ED drug, tadalafil, from which it differs in having one less methyl group. Their fragmentation patterns are, therefore, expected to be similar. The tandem MS spectra were subsequently interrogated to verify the suspected ED drug. As seen in Fig. 1C, nortadalafil exhibits two product ions at *m/z* 135.0431 and *m/z* 169.0745 within ±20 ppm of mass error, corresponding to those of tadalafil fragmentation patterns. These findings indicated that nortadalafil is indeed a suspected analyte belonging to the tadalafil group of analogues.

Fig. 2 summarises the identification of target analytes and the detection of suspected analytes in all food samples. The majority of the adulterants were those of the approved ED drugs such as sildenafil, vardenafil, and tadalafil. Top of the list is tadalafil, identified in five samples as a sole adulterant, also in

combination with other ED drugs in another eight samples. Sildenafil and vardenafil were identified in ten and one samples, respectively, mostly in combination with other ED drugs. This study also identified unapproved analogues of ED drugs as follows: thiosildenafil (ten samples); thiodimethylsildenafil (eight samples); dimethylsildenafil (five samples); propoxyphenyl-thiohydroxyhomosildenafil (two samples); and propoxyphenyl-thiodimethylsildenafil (one sample) with up to five different adulterants per sample.

Briefly, only 23 samples were quantified, excluding samples HCD002 and PDM009. The target analytes were quantified from 1.8 to 148.4 mg per recommended intake, as specified on the products' labels. The quantification levels were divided into subtherapeutic, therapeutic, and supratherapeutic based on the recommended dose of the approved ED drugs (i.e. 25–100 mg for sildenafil and 5–20 mg for vardenafil and tadalafil) [29]. In summary, four samples were quantified at subtherapeutic level, while another eight and 11 samples were quantified at therapeutic and supratherapeutic levels, respectively. The supratherapeutic levels of tadalafil in ten samples were of grave concern, especially for sample PDM005 that was quantified at 103.9 mg per recommended intake, which exceeded five times the tadalafil maximum daily dose. Tadalafil possesses the longest duration of action among all of the approved ED drugs [30]. Therefore, there is a strong probability of developing delayed side effects due to extended exposure of tadalafil in the systemic circulation [31]. At supratherapeutic level, tadalafil might lead to an even higher incidence of side effects, posing severe health and life-threatening risks to consumers.

4. Conclusion

In the present work, LC-HRMS-based method was utilised for accurate and precise analysis of ED drugs and their analogues in selected food matrices. The modified QuEChERS extraction provided complete coverage of target analytes, exhibiting insignificant ME and satisfactory RE for almost all target analytes in the selected food matrices. The modified QuEChERS extraction was subsequently validated for each food matrix and applied to determine ED drugs in 25 food samples claiming to enhance male sexual performance. The targeted analysis, together with suspected-target and non-targeted screenings of an LC-HRMS, revealed 24 adulterated food samples with 11 of them quantified at supratherapeutic levels. The comprehensive strategies discussed in this study would be beneficial to curb the widespread adulteration of ED drugs in food products, and more importantly, to safeguard the consumers from potentially short- and long-term health problems, which could lead to life-threatening crises.

CRediT authorship contribution statement

Ahmad Yusri Mohd Yusop: Conceptualisation, Methodology, Validation, Formal analysis, Investigation, Resources, Writing - Original Draft, Visualization. **Linda Xiao:** Conceptualisation, Supervision, Writing - Review & Editing. **Shanlin Fu:** Conceptualisation, Supervision, Writing - Review & Editing, Funding acquisition.

Declaration of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this study.

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Table 1: Contents of the blank food matrices based on the products' label.

No.	Matrix	Listed ingredients on the label
1	Powdered drink mix (PDM)	Citric acid, calcium phosphate, maltodextrin, ascorbic acid, natural and artificial flavour, blue 1, tocopherol, and preservatives.
2	Honey (HNY)	100% pure Australian eucalyptus and ground flora honey.
3	Jelly (JLY)	Water, sugar, gelling agents, acidity regulators, anthocyanins, and natural flavours.
4	Hard candy (HCD)	Sugar, glucose syrup, water, natural herbs extract, natural and artificial flavour, caramel colouring, and menthol.
5	Sugar-coated chewing gum (CWG)	Sorbitol, gum base, humectant, mannitol, flavour, sweetener, emulsifier, antioxidant, and phenylalanine.

Table 2: Chemical formula, retention time (RT), accurate mass of protonated molecule ($[M+H]^+$) precursor ion, product ions, coefficient of determination (r^2), and limit of detection (LOD) of 23 targeted ED drugs.

No.	Target analytes	Chemical formula	RT (min)	Accurate mass of $[M+H]^+$ (*m/z*)	Product ion 1 (*m/z*)	Product ion 2 (*m/z*)	r^2	LOD (ng/mL)
1	Desmethylcarbodenafil	$C_{23}H_{30}N_6O_3$	8.77	439.2452	311.1139	339.1452	0.9989	10
2	Carbodenafil	$C_{24}H_{32}N_6O_3$	9.24	453.2609	311.1139	339.1452	0.9994	10
3	N-desethylacetildenafil	$C_{23}H_{30}N_6O_3$	9.68	439.2452	325.1295	297.1346	0.9993	40
4	Acetildenafil	$C_{25}H_{34}N_6O_3$	10.64	467.2765	297.1346	127.1230	0.9971	10
5	Hydroxyvardenafil	$C_{23}H_{32}N_6O_5S$	10.81	505.2228	312.1581	151.0866	0.9996	10
6	Dimethylacetildenafil	$C_{25}H_{34}N_6O_3$	11.15	467.2765	297.1346	127.1230	0.9966	10
7	Vardenafil	$C_{23}H_{32}N_6O_4S$	11.48	489.2279	312.1581	151.0866	0.9990	60
8	Sildenafil	$C_{22}H_{30}N_6O_4S$	13.34	475.2122	283.1190	100.0995	0.9995	10
9	Homosildenafil	$C_{23}H_{32}N_6O_4S$	13.91	489.2279	283.1190	113.1073	0.9988	40
10	Dimethylsildenafil	$C_{23}H_{32}N_6O_4S$	14.66	489.2279	283.1190	113.1073	0.9990	20
11	Propoxyphenyl-hydroxyhomosildenafil	$C_{24}H_{34}N_6O_5S$	15.68	519.2384	129.1022	283.1190	0.9995	10
12	Udenafil	$C_{25}H_{36}N_6O_4S$	15.90	517.2592	112.1121	283.1190	0.9995	10
13	Propoxyphenyl-sildenafil	$C_{23}H_{32}N_6O_4S$	16.07	489.2279	100.0995	283.1190	0.9975	10
14	Hydroxythiovardenafil	$C_{23}H_{32}N_6O_4S_2$	18.33	521.1999	167.0637	328.1352	0.9995	40

15	Tadalafil	$C_{22}H_{19}N_3O_4$	20.86	390.1448	135.0441	169.0760	0.9960	40
16	Mirodenafil	$C_{27}H_{35}N_9O_5S_2$	21.45	532.2588	312.1343	296.1394	0.9995	10
17	Mutaprodenafil	$C_{26}H_{37}N_5O_5S$	21.62	630.2275	113.1073	142.0070	0.9976	10
18	Thiosildenafil	$C_{22}H_{30}N_6O_3S_2$	24.74	491.1894	100.0995	299.0961	0.9992	30
19	Thiohomosildenafil	$C_{23}H_{32}N_6O_3S_2$	25.74	505.2050	299.0961	113.1073	0.9982	60
20	Dithiodesmethylcarbodenafil	$C_{23}H_{30}N_6OS_2$	26.08	471.1995	343.0682	371.0995	0.9961	10
21	Thiodimethylsildenafil	$C_{23}H_{32}N_6O_3S_2$	26.50	505.2050	113.1073	299.0961	0.9991	70
22	Propoxyphenyl-thiohydroxyhomosildenafil	$C_{24}H_{34}N_6O_4S_2$	27.26	535.2156	129.1022	299.0961	0.9991	20
23	Propoxyphenyl-thiodimethylsildenafil	$C_{24}H_{34}N_6O_3S_2$	30.09	519.2207	113.1073	299.0961	0.9991	10

Table 3: The identification of target analytes and the detection of suspected analytes in 25 food samples.

Sample	Target analytes identified / *suspected analytes detected (average weight per recommended intake in mg)	Total average weight per recommended intake in mg	Quantification level
PDM001	1. Sildenafil (0.3) 2. Tadalafil (87.7)	88.0	Supratherapeutic
PDM002	1. Propoxyphenyl-thiohydroxyhomosildenafil (2.2)	2.2	Subtherapeutic
PDM003	1. Tadalafil (80.9) 2. Thiodimethylsildenafil (1.1) 3. Thiosildenafil (7.5)	89.5	Supratherapeutic
PDM004	1. Tadalafil (31.1)	31.1	Supratherapeutic
PDM005	1. Tadalafil (103.9) 2. Thiosildenafil (<LOQ)	103.9	Supratherapeutic
PDM006	1. Propoxyphenyl-thiohydroxyhomosildenafil (0.5) 2. Thiodimethylsildenafil (0.4) 3. Thiosildenafil (4.6)	5.5	Subtherapeutic
PDM007	1. Dimethylsildenafil (0.5) 2. Sildenafil (0.1) 3. Thiodimethylsildenafil (68.4) 4. Thiosildenafil (10.1)	79.1	Therapeutic
PDM008	1. Tadalafil (38.5)	38.5	Supratherapeutic
PDM009	Not detected	Not applicable	Not applicable

PDM010	1. Dimethylsildenafil (0.3) 2. Tadalafil (20.9) 3. Thiodimethylsildenafil (33.9) 4. Thiosildenafil (6.0) 5. Sildenafil (<LOQ)	61.1	Supratherapeutic
PDM011	1. Tadalafil (9.6) 2. Thiodimethylsildenafil (24.6) 3. Thiosildenafil (<LOQ)	34.2	Therapeutic
PDM012	1. Dimethylsildenafil (0.1) 2. Tadalafil (28.9) 3. Thiodimethylsildenafil (13.7) 4. Thiosildenafil (0.2) 5. Sildenafil (<LOQ)	42.9	Supratherapeutic
PDM013	1. Sildenafil (4.6) 2. Tadalafil (23.5)	28.1	Supratherapeutic
PDM014	1. Sildenafil (33.7) 2. Tadalafil (18.5)	52.2	Therapeutic
PDM015	1. Thiodimethylsildenafil (55.3) 2. Dimethylsildenafil (<LOQ)	55.3	Therapeutic
PDM016	1. Tadalafil (79.4)	79.4	Supratherapeutic
HNY001	1. Sildenafil (2.2) 2. Thiosildenafil (36.2)	38.4	Therapeutic
HNY002	1. Sildenafil (0.9) 2. Thiosildenafil (60.9)	61.8	Therapeutic
HNY003	1. Tadalafil (3.7)	3.7	Subtherapeutic

HNY004	1. Propoxyphenyl-thiodimethylsildenafil (33.4) 2. Thiodimethylsildenafil (3.8) 3. Dimethylsildenafil (<LOQ) 4. Propoxyphenyl-dimethylsildenafil*	37.2	Therapeutic
JLY001	1. Vardenafil (17.9)	17.9	Therapeutic
JLY002	1. Sildenafil (148.4)	148.4	Supratherapeutic
HCD001	1. Tadalafil (49.8)	49.8	Supratherapeutic
HCD002	1. Nortadalafil*	Not applicable	Not applicable
CWG001	1. Sildenafil (0.3) 2. Thiosildenafil (1.5)	1.8	Subtherapeutic

Abbreviations: PDM, powdered drink mix; HNY, honey; JLY, jelly; HCD, hard candy; CWG, sugar-coated chewing gum; LOQ, limit of quantification; *suspected analyte.

Figure captions

Fig. 1 The suspected-target screening of sample HCD002 with (A) extracted ion chromatogram of nortadalafil, matched using the “Find Compounds by Formula (FBF)” technique; (B) full MS scan; and (C) tandem MS spectra at averaged collision energies showing the proposed fragmentation patterns.

Fig. 2 Results summary of erectile dysfunction drugs in adulterated food samples; with identification of target analytes and detection of suspected analytes.

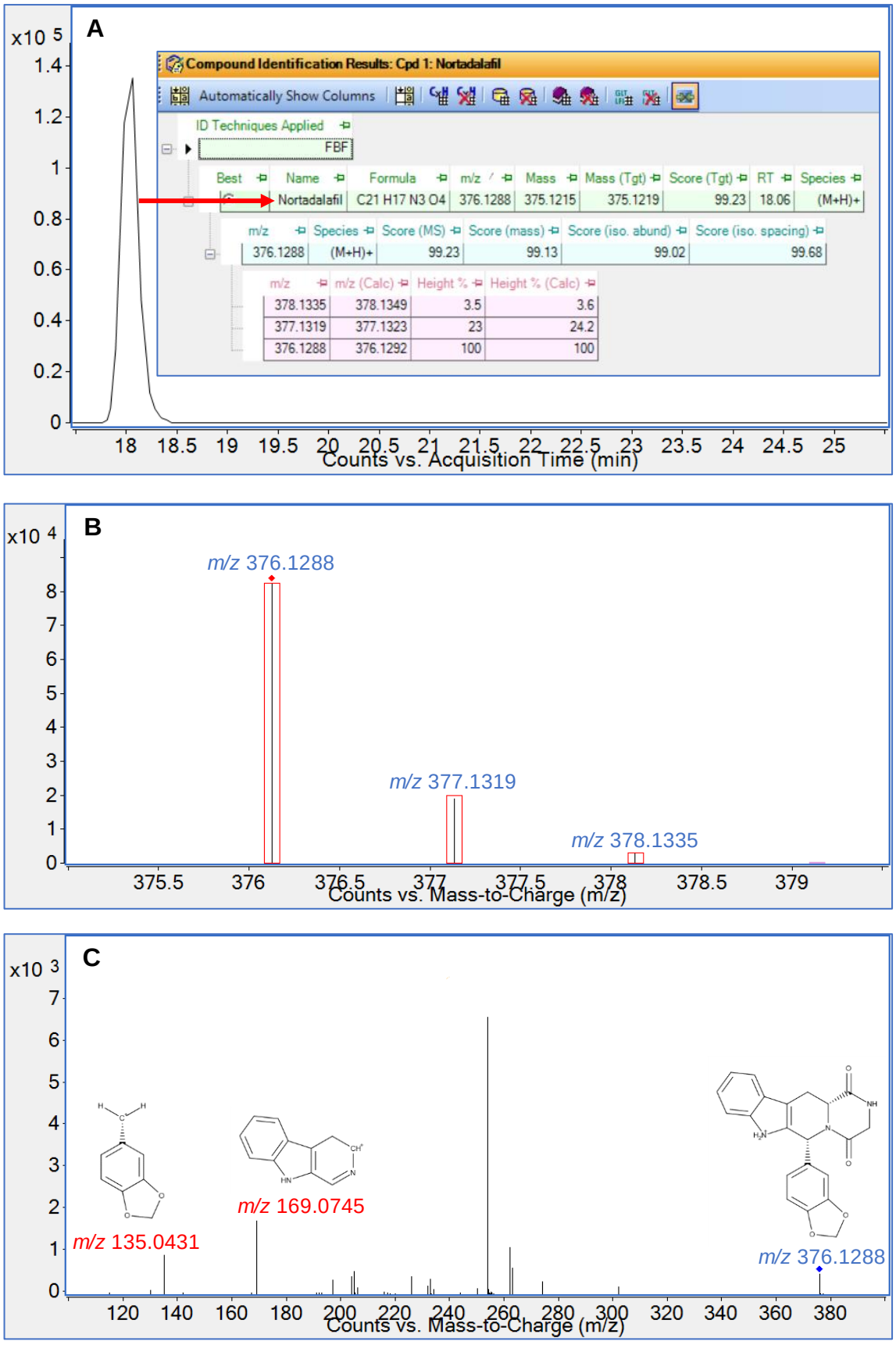
A
x10 5
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Compound Identification Results: Cpd 1: Nortadalafil
Automatically Show Columns
ID Techniques Applied
FBF
Best | Name | Formula | m/z | Mass | Mass (Tgt) | Score (Tgt) | RT | Species
Nortadalafil | C21 H17 N3 O4 | 376.1288 | 375.1215 | 375.1219 | 99.23 | 18.06 | (M+H)+
m/z | Species | Score (MS) | Score (mass) | Score (iso. abund) | Score (iso. spacing)
376.1288 | (M+H)+ | 99.23 | 99.13 | 99.02 | 99.68
m/z | m/z (Calc) | Height % | Height % (Calc)
378.1335 | 378.1349 | 3.5 | 3.6
377.1319 | 377.1323 | 23 | 24.2
376.1288 | 376.1292 | 100 | 100
18 18.5 19 19.5 20 20.5 21 21.5 22 22.5 23 23.5 24 24.5 25
Counts vs. Acquisition Time (min)
B
x10 4
8
7
6
5
4
3
2
1
0
m/z 376.1288
m/z 377.1319
m/z 378.1335
375.5 376 376.5 377 377.5 378 378.5 379
Counts vs. Mass-to-Charge (m/z)
C
x10 3
7
6
5
4
3
2
1
0
m/z 135.0431
m/z 169.0745
m/z 376.1288
120 140 160 180 200 220 240 260 280 300 320 340 360 380
Counts vs. Mass-to-Charge (m/z)

Fig.1

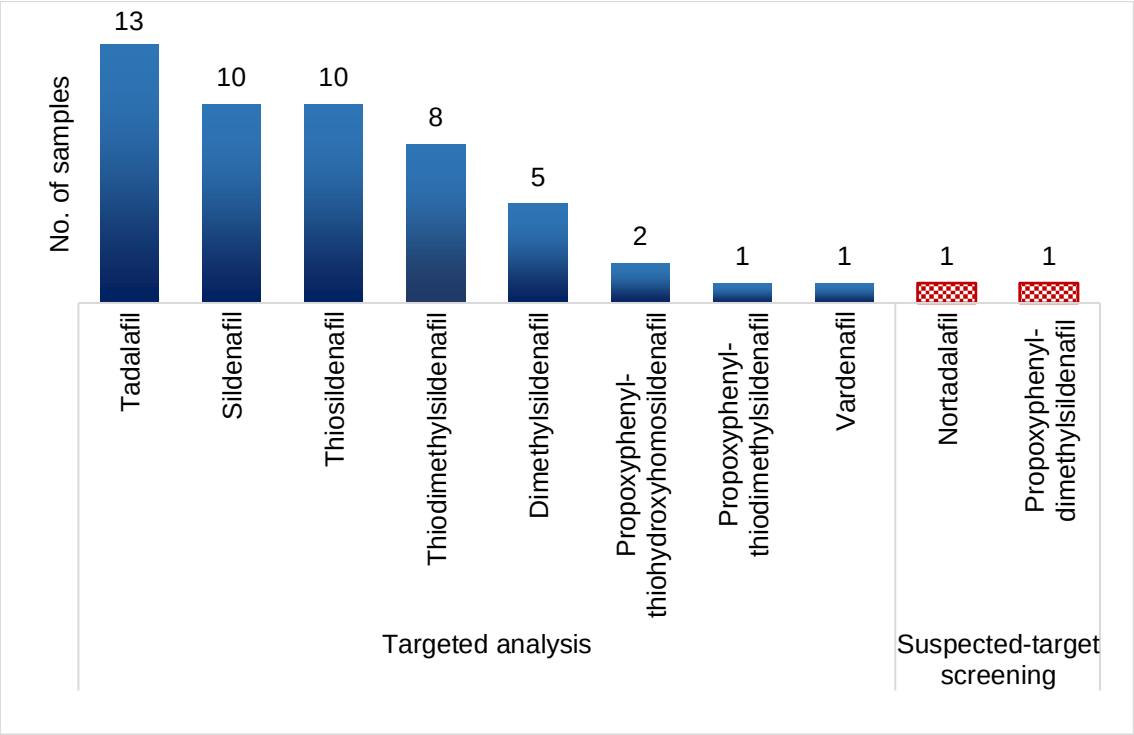
No. of samples
13
10
10
8
5
2
1
1
1
1
Tadalafil
Sildenafil
Thiosildenafil
Thiodimethylsildenafil
Dimethylsildenafil
Propoxyphenyl-thiohydroxyhomosildenafil
Propoxyphenyl-thiodimethylsildenafil
Vardenafil
Nortadalafil
Propoxyphenyl-dimethylsildenafil
Targeted analysis
Suspected-target screening

Fig. 2